POSTER SESSION

1001 Basic Mechanisms of Myocardial Protection

Sunday, March 17, 2002, 9:00 a.m.-11:00 a.m.
Georgia World Congress Center, Hall G
Presentation Hour: 10:00 a.m.-11:00 a.m.

1001-27 Metallothionein Elicits Second Window of Myocardial Protection in Rabbit Hearts Through Mitogen-Activated Protein Kinase Mechanism

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Background: Ischemic preconditioning (IPC) of myocardium displays a bimodal time course. Early cardioprotection wanes rapidly and is succeeded by a delayed phase. This "second window" of protection (SWOP) lasts up to 72 hours. Metallothionein, a stress-induced protein, is an important endogenous mediator exerting acute protective effects in the ischemic myocardium. We examined whether endogenous metallothionein could evoke SWOP (delayed myocardial protection).
Methods and Results: Rabbits (n=18) were subjected to IPC with 4 cycles of 5-min regional ischemia each followed by 10-min reperfusion. Twenty-four hours later, animals were subjected to sustained 45 min. ischemia followed by 60 min reperfusion (I/R). Parallel groups were sham control (n=10), or I (45min)/R (60 min)(n=10). Activity of mitogen-activated protein kinase (MAPK) in IPC myocardium was increased 10 to 12 times immediately after IPC (P<0.01 vs. sham control), and returned to control 24 hours later. In these experiments, myocardial metallothionein gene expression (Northern and Western blots) was increased 3 to 7 times at 24 hours after IPC compared with that immediately after IPC as well as the sham control group (both P<0.01). Pretreatment of rabbits with PD098059 inhibited MAPK activation as well as metallothionein gene expression in response to IPC (P<0.01). Myocardial infarct size was assessed by TTC staining and expressed as percent of risk zone. Cardiac function was measured by monitoring ±dp/dt. IPC reduced infarct size (34 ±7% to 23 ±3%, a 48% reduction, n = 9, P<0.01). IPC also improved dp/dt (21938±1663mmHg to 33609±3178mmHg, a 53% reduction, n=9, P<0.01). IPC also reduced lipid peroxidation and LDH release compared with that in non-IPC (both P<0.01). Importantly, we found that the myocardial metallothionein content was positively correlated to the reduction in infarct size and improvement in cardiac function.
Conclusions: The upregulation of metallothionein gene expression induced by IPC plays an important role in the delayed protection of ischemic myocardium. The expression of metallothionein is mediated by the activation of MAPK in response to IPC.

1001-28 Matrix Metalloproteinase Inhibition Attenuates Postinfarction Left Ventricular Remodeling

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Myocardial matrix metalloproteinase activity is increased following experimental MI and may play a role in post-infarction LV remodeling. However, effects of matrix metalloproteinase inhibition (MMPi) on post-infarction LV remodeling and systolic function remain incompletely understood. Accordingly, MMPi was studied in a well-characterized ovine post-infarction model. One day after anteroapical MI by coronary ligation, 14 sheep were randomized to either no therapy (Control, n=8) or non-selective MMPi (PD166793 - Pfizer, Inc.; n=6). Magnetic resonance imaging (GE/CVi) was performed at baseline and at 8-weeks post-MI to quantify changes in LV end-systolic volume index (ESVi), end-diastolic volume index (EDVi), ejection fraction (EF), and mass index (LVMi). **Results:** Infarct size as a % of LV mass was similar for MMPi vs Control: 17±1 vs 17±2 %. Left atrial pressure at 8-weeks post-MI was not different: 9±2 vs 8±2 mmHg. Baseline LV EDVi, ESVi, EF, and LVMi were similar between groups. There was significant limitation of LV remodeling and greater preservation of EF at 8-weeks post-MI with MMPi compared to Control (see Table). At 8-weeks post-MI, EDVi and ESVi were significantly lower with MMPi compared to Control: 1.8±0.1* vs 2.4±0.2 ml/kg and 1.1±0.1* vs 1.6±0.2 ml/kg (*p<0.04). **Conclusions:** Non-selective MMPi attenuated post-infarction LV remodeling in this large animal model. Unlike smaller animal models, however, MMPi also better preserved global LV systolic function following MI.

Change in LV Remodeling Parameters (*p<0.02 vs Control)

	EDVi (ml/kg)	ESVi (ml/kg)	EF (%)	LVMi (g/kg)
MMPi	+0.4±0.1*	+0.4±0.1*	-14±2*	+0.1±0.1
Control	+0.8±0.1	+0.9±0.1	-21±2	+0.2±0.1

1001-29 Phosphatidylinositol 3-Kinase/Akt Pathway Contributes to Ischemic Preconditioning in Isolated Rabbit Heart

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Background: Several growth factors and cytokines have been shown to trigger cell survival signaling via activation of the phosphatidylinositol 3-kinase (PI3 kinase)/Akt pathway. The present study examined whether activation of PI3 kinase/Akt plays a role in the cardioprotective mechanism of ischemic preconditioning (PC). **Methods**: Adult rabbit hearts perfused in the Langendorff fashion were subjected to 30 min of global ischemia followed by 2 h of reperfusion. PC was achieved by pre-exposing the heart to 5 min of global ischemia followed by 10 min of reperfusion before the standard ischemia-reperfusion treatment. To evaluate the role of phospho-Akt (the active form of Akt) in the cardioprotection of PC, the PI3-kinase inhibitor wortmannin was used to deplete phospho-Akt. Wortmannin (100 nM) infusion was started 5 min before the preconditioning ischemia and continued until the index ischemia. Infarct size was determined by computer morphometry of TTC stained sections. Phospho-Akt (Ser473) levels were determined by Western blot analysis in a separate series of hearts, in which serial biopsies were taken at baseline and at 5, 10, 20, 30 min of ischemia. **Results**: The PC hearts exhibited smaller infarct size (24± 5%, n=6) than in the control group (50± 5%, n=6). In the PC+Wortmannin group, the infarct size was 53± 10% (n=6). In the control hearts, ischemia caused a progressive loss of phospho-Akt, such that at 30 min of ischemia the phospho-Akt level was only 14± 2% of baseline. PC significantly attenuated the ischemia-induced loss of phospho-Akt, such that at 30 min of ischemia the phospho-Akt level was maintained at 39± 8% of baseline ($p<0.05$ *vs* control group). PC+Wortmannin group showed complete depletion of phospho-Akt. **Conclusion**: Myocardial ischemia is associated with a marked depletion of phospho-Akt. PC preserves phospho-Akt level in the ischemic myocardium and protects the heart from ischemia/reperfusion-induced cell death. The cardioprotective effect of PC could be completely abolished by depletion of phospho-Akt via inhibition of PI3-kinase. Thus, activation of the PI3-kinase/Akt pathway appears to be an important mechanism mediating PC-induced cardioprotection.

1001-30 Endocardial Cryotherapy Induces Marked Arteriogenesis in Normal Porcine Myocardium

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Background: Percutaneous Myocardial Revascularization (PMR) using heat-based catheters (radiofrequency, laser) has been studied extensively in recent clinical trials with controversial results. However, the myocardial response may be substantially different with cold therapy. Therefore, we studied the neovascular response of myocardial tissue following exposure to cryo-energy in a non-ischemic porcine model.
Methods: Cryo-energy was applied to the endocardium using a 7Fr Freezor™ focal cryocatheter (CryoCath Technologies) in the left ventricle of 25 normal swine. Up to 8 discreet treatments of varying temperature (-30 to -75°C) and time (30 to 120 sec) were performed. Hearts were harvested at 4 weeks, the coronary arteries were perfusion fixed and injected with a barium gelatin.
Results: Histological analysis revealed clearly demarcated lesions extending into the myocardial wall. Capillary, arteriole and muscular artery vessel densities were assessed in normal myocardium, within the cryo-lesion and at 1 mm margin around the cryo-lesion using lectin, actin and elastin stains. While capillary density (n/mm2) decreased in the 1mm rim (1492 ± 679, P<0.001) and in the lesion (218 ± 183, P<0.001) as compared to control myocardium (3154 ± 792), arteriole density increased significantly in the 1mm rim (7.02 ± 4.87, P<0.001) and in the lesion (7.62 ± 4.81, P<0.001) as compared to control (4.62 ± 1.36). Muscular artery density also increased in the 1mm region (0.34 ± 0.37, P<0.001) and in the lesion (0.35 ± 0.30, P<0.001) vs. control (0.05 ± 0.03). Arteriolar density correlated with lesion volume indicating possible release of growth factors or cytokines during lesion formation and subsequent healing response. Barium gelatin infusion confirmed communication of arterioles and muscular arteries with the epicardial coronary arteries.
Conclusion: Endocardial cryoapplications in the range of -30°C to -75°C can induce marked arteriogenesis within the myocardium of non-ischemic pigs. This energy source may represent a promising alternative for patients suffering from myocardial ischemia with limited therapeutic options.

1001-47 Adenosine Administered From the Beginning of Reperfusion Protected Myocardial Stunning by Activation of A1 Receptors and K+ATP Channels

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Background: There is experimental evidence that adenosine (ADO), added from the beginning of reperfusion attenuates systolic and diastolic alterations in myocardial stunning. The aim was to determine the participation of A1 receptors and ATP K+ channels acting as mediators of the above effects. Methods: Isolated rabbit hearts were used and four groups were formed. Group 1 (G1, n=14) was subjected to 15 min of global ischemia followed by 30 min of reperfusion. In Group 2 (G2, n=12) the protocol of G1 was repeated but adding ADO (0.3 mg/kg/min) to the perfusate from the beginning of reperfusion. In Group 3 (G3, n=12) the protocol of G2 was repeated, but DPCPX (200 nM), a selective blocking agent of A1 receptors, was administered in presence of ADO. In Group 4 (G4, n=9) the protocol of G2 was repeated, but glibenclamide (0.3 μM), a blocking agent of ATP K+ channels, was administered in presence of ADO. Left ventricular developed pressure (LVDP, mmHg) and left ventricular end diastolic pressure (LVEDP, mmHg) (diastolic stiffness) were calculated, and the infarct size measured. Results: Infarct size was not different between G1 and G2 (2.75±0.8 and 4.35±1.6; NS), but it was bigger in G3 and G4 (10.7±1.3 and 12.7±2.2; p<0.05) compared to G1. X±SEM *:p<0.05 vs G1. Conclusions: ADO administered from the beginning of reperfusion attenuated the alteration of contractile state and diastolic stiffness by the activation of A1 receptors and K+ATP channels. The ADO protection was independent of the modification in the infarct size.